A method of characterizing fluorescent molecules or other particles using generating functions

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This invention relates to the field of fluorescence spectroscopy, and more particularly to a method for determining characteristic physical quantities of fluorescent molecules or other particles present in a sample.

US patent 4,198,571 discloses a scanning microscope including first and second focussing means arranged confocally, at least one focussing means being of annular form, a coherent radiation detection means arranged to receive radiation from one focussing means, and scanning means to scan an object in the focal plane.

US Patent 5,866,911 discloses that in scanned optical systems such as confocal laser microscopes wherein a beam of light is focused to a spot in a specimen to excite a fluorescent species or other excitable species in the spot, the effective size of the excitation is made smaller than the size of the spot by providing a beam of light of wavelength adapted to quench the excitation of the excitable species, shaping this second beam into a pattern with a central intensity minimum, and overlapping this central minimum with the central intensity maximum of the focused spot, so that within the spot the intensity of quenching light increases with distance from the center of the spot, thereby preferentially quenching excitation in the peripheral parts of the spot, and thereby reducing the effective size of the excitation and thus improving the resolution of the system.

The primary data of an experiment in fluorescence correlation spectroscopy (FCS, a prior art technique) is a sequence of photon counts detected from a microscopic measurement volume. An essential attribute of the fluorescence correlation analysis is the calculation of the second order autocorrelation function of photon detection. This is a way how a stochastic function (of photon counts) is transformed into a statistical function having an expected shape, serving as a means to estimate some parameters of the sample. However, the calculation of the autocorrelation function is not the only way for extracting information about the sample from the sequence of photon counts. Further approaches are based

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